

10/594908

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SEQUENCE LISTING

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Piao, Jinhua
Lin, Qing

<120> A GENETIC ENGINEERING RECOMBINANT ANTI-CEA, ANTI-CD3
AND ANTI-CD28 SINGLE-CHAIN TRI-SPECIFIC ANTIBODY

<130> 11774-006-999 (I040179)

<150> PCT/CN2005/000408
<151> 2005-03-29

<150> CN 200410032158.3
<151> 2004-04-01

<160> 58

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 251
<212> PRT
<213> Murine

<220>
<223> murine anti-CEA single chain fragment of variable region
contained in CEA-scTsAb

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Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala
1 5 10 15
Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Asp Tyr
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Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Leu Pro Gly Ser Gly Arg Thr Asp Tyr Asn Glu Arg Phe
50 55 60
Lys Gly Lys Ala Thr Phe Thr Gly Asp Val Ser Ser Asn Thr Ala Tyr
65 70 75 80
Met Lys Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95
Ala Thr Gly Thr Thr Pro Phe Gly Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110
Thr Val Ser Ala Thr Ser Thr Pro Ser His Asn Ser His Gln Val Pro
115 120 125
Ser Ala Gly Gly Pro Thr Ala Asn Ser Gly Ser Arg Asp Ile Val Leu
130 135 140
Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr
145 150 155 160
Ile Ser Cys Arg Ala Ser Gln Ser Val Ser Thr Ser Ser Tyr Thr Tyr
165 170 175
Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
180 185 190
Lys Tyr Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly
195 200 205

Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Asn	Ile	His	Pro	Val	Glu	Glu
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Glu	Asp	Thr	Ala	Tyr	Tyr	Tyr	Cys	Gln	His	Ser	Trp	Glu	Ile	Pro	Arg
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Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys					
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<210> 2
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<220>
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 contained in CEA-scTsAb

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Ser	Met	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr	Gly	Tyr
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Thr	Met	Asn	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Asn	Leu	Glu	Trp	Met
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Gly	Leu	Ile	Asn	Pro	Tyr	Lys	Gly	Val	Ser	Thr	Tyr	Asn	Gln	Lys	Phe
	50					55					60				
Lys	Asp	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
65					70					75					80
Met	Glu	Leu	Leu	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys
			85						90					95	
Ala	Arg	Ser	Gly	Tyr	Tyr	Gly	Asp	Ser	Asp	Trp	Tyr	Phe	Asp	Val	Trp
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Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	Ser	Ala	Ser	Leu	Gly	Asp	Arg	Val
145					150					155					160
Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Arg	Asn	Tyr	Leu	Asn	Trp
			165						170					175	
Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Val	Lys	Leu	Leu	Ile	Tyr	Tyr	Thr
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Ala	Thr	Tyr	Phe	Cys	Gln	Gln	Gly	Asn	Thr	Leu	Pro	Trp	Thr	Phe	Ala
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gtgaaacagc gtccgggtca cggcctggaa tggatcggtg aaatcctgcc gggcagcggc 180
cgtaccgact acaacgaacg tttcaaagcg aaagcgacct tcaccggcga cgtttctagc 240
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gcgaccggca ccaccccggt cggttactgg ggtcagggca ccctggttac cgtttccgcg 360
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<210> 4
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<220>
<223> CEA-scTsAb sequence

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Phe Ser Asp Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly
 35          40          45
Leu Glu Trp Ile Gly Glu Ile Leu Pro Gly Ser Gly Arg Thr Asp Tyr
 50          55          60
Asn Glu Arg Phe Lys Gly Lys Ala Thr Phe Thr Gly Asp Val Ser Ser
 65          70          75          80
Asn Thr Ala Tyr Met Lys Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala
 85          90          95
Val Tyr Tyr Cys Ala Thr Gly Thr Thr Pro Phe Gly Tyr Trp Gly Gln
100          105          110
Gly Thr Leu Val Thr Val Ser Ala Thr Ser Thr Pro Ser His Asn Ser

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Leu	Glu	Cys	Leu	Gly	Val	Ile	Trp	Gly	Gly	Gly	Thr	Asn	Tyr	Asn	Ser
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Ala	Leu	Met	Ser	Arg	Arg	Val	Thr	Ser	Ser	Asp	Asp	Thr	Ser	Lys	Asn
625					630					635					640
Gln	Phe	Ser	Leu	Lys	Leu	Ser	Ser	Val	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			645						650					655	
Ala	Arg	Ser	Tyr	Tyr	Ser	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	
		660					665					670			
Val	Thr	Val	Ser	Ser	Gly	Thr	Glu	Gln	Lys	Leu	Ile	Ser	Glu	Glu	Asp
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<400> 5
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<210> 6
 <211> 59
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<400> 6
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<210> 7
 <211> 59
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<400> 7
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<210> 8
 <211> 59
 <212> DNA
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<220>
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<400> 8
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<210> 9
 <211> 59
 <212> DNA
 <213> Artificial Sequence

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 <400> 9
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 <210> 10
 <211> 59
 <212> DNA
 <213> Artificial Sequence

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 <400> 10
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 <210> 11
 <211> 59
 <212> DNA
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 <400> 11
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 <210> 12
 <211> 59
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 <400> 12
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 <210> 13
 <211> 64
 <212> DNA
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 <400> 13
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 tcgg 64

<210> 14
 <211> 59
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 <400> 14
 atcgagctca tgtacccgcg cggtaacgct agcgaacaaa aactcatctc agaagagga 59

 <210> 15
 <211> 59
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 <400> 15
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 <400> 16
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 <400> 17
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 <210> 18
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 <400> 18
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 <400> 19
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 <400> 20
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 <400> 21
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 <210> 22
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 <400> 22
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 <210> 23
 <211> 15
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 anti-CEA monoclonal antibody

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<210> 24
 <211> 20
 <212> DNA
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 <400> 24
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 <210> 25
 <211> 58
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 <212> DNA
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 <400> 46
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<210> 49
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 <400> 51
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 <210> 52
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 <400> 52
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 <210> 53
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 <213> Artificial Sequence

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 <400> 53
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 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys

20

25

<210> 54
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<213> Artificial Sequence

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<400> 54
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Ser Thr Pro Thr Pro Val Glu Val Ser
20 25

<210> 55
<211> 24
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<213> Artificial Sequence

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<400> 55
Ala Leu Glu Val Asp Glu Thr Tyr Val Pro Lys Glu Phe Asn Ala Glu
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Thr Phe Thr Phe His Ala Asp Ile
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<210> 56
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<212> PRT
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<223> cmyc tag

<400> 56
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1 5 10

<210> 57
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> His tag

<400> 57
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<210> 58
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<220>

<223> nucleotide sequence showing multiple cloning sites (fig 3)

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aataaggatc cgtcgag                                     437
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